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ELIMINATION AND TOXICITY OF [illegible] IN
NEPHRECTOMIZED RABBITS

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R. E. DOOLITTLE, ACTING CHIEF OF BUREAU.

THE ELIMINATION OF CAFFEIN:

AN EXPERIMENTAL STUDY ON HERBIVORA AND CARNIVORA.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., March 25, 1912.

Sir: I have the honor to present for your approval a report of an experimental study on the elimination of caffein, conducted in the Pharmacological Laboratory of the Division of Drugs. This work is in continuation of the studies reported in Bulletin 148, on the toxicity of caffein, and is of special interest in connection with certain problems arising under the administration of the food and drugs act, as well as of general scientific and professional value. I recommend that this manuscript be published as Bulletin 157 of the Bureau of Chemistry.

Respectfully, R. E. Doolittle,
Acting Chief.

Hon. James Wilson,
Secretary of Agriculture.

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THE ELIMINATION OF CAFFEIN.

AN EXPERIMENTAL STUDY ON HERBIVORA AND CARNIVORA.

INTRODUCTION.

As pointed out in a previous report (16),[1] the action of caffein has been extensively studied during the past eighty years. Not until the last decade of the nineteenth century, however, has its fate in the body been made the subject of serious investigation. The appearance in the nineties of the work of Albanese (1), of Bondzynski and Gottlieb (3), and of Krüger and Schmidt (7), in which the character of the decomposition products of caffein and of theobromin was established for the first time, marks, therefore, the beginning of accurate information on the metabolism of caffein. These investigations, however, concerned themselves mainly with the transformation products of caffein and allied substances, while the extent to which it was eliminated unchanged received but little attention. The study of the elimination of caffein was not altogether neglected, however. As shown in the following section, a number of attempts have been made to investigate this problem.

HISTORICAL REVIEW OF THE LITERATURE.

An examination of the literature on caffein showed that the earliest studies were those published by Lehmann (9), who reported negative results. According to Hammersten (6), caffein is found in the urine after drinking tea or coffee as well as after the ingestion of pure caffein. His conclusions were based on the presence of crystals which he extracted from the urine and on the murexid test. Draggendorff (5) examined the urine of individuals after drinking tea and coffee, but was unable to find caffein. Aubert (2) analyzed the urines of individuals who had taken caffein by mouth, and claimed to have obtained positive results with the chlorin test.

Experimental studies on animals were made for the first time by Strauch (14). According to his observations caffein was present in the urine and bile of cats, dogs, rabbits, and guinea pigs after its administration by mouth in doses of 0.25 to 0.5 gram. He examined the urine at various intervals and stated that he found the alkaloid in some cases two hours after it was given. For the separation of caffein the urine was made alkaline with ammonium hydroxid and extracted

[1] Numbers in parenthesis refer to Bibliography, on p. 23.

with chloroform. It was identified by the use of the murexid test and by precipitation with phosphomolybdic acid. Similar results were reported by Schwenger (13) in individuals drinking coffee, but his conclusions were based on the observation of a precipitate formed by iodin and potassium iodid.

Schutzkwehr (12), who studied the elimination of caffein in dogs and rabbits, reported its presence in the urine of a dog which received 4 grams and of a rabbit which received 0.2 gram of caffein. He stated that he recovered 6 per cent of it in the urine of the rabbit and found a small quantity in the feces. Schneider (11) examined the urine of cats and human urine for caffein at different intervals after its administration. He also studied the elimination of caffein in the cat after administration by different methods, as well as the influence of the size of the dose on elimination. When 30 to 100 mg of caffein were given by mouth to these animals some of it was found in the urine at the end of twelve hours, and caffein was still present even after twenty-four hours. When smaller doses were administered none could be detected in the urine. There was apparently a difference in the behavior of caffein in this regard when it was given subcutaneously, as Schneider (11) reported that he could not obtain any caffein from the urine after the subcutaneous injection of 30 mg, but he could detect its presence when 50 mg were thus administered, also stating that he could find it in the urine after two hours.

In an experiment on a dog to which 100 mg of caffein were administered Maly and Andreasch (10) recovered 66 mg in the urine by chloroform extraction. These investigators maintained that caffein is not decomposed in the body.

The subject was studied more extensively by Rost, who made experiments on the elimination of caffein in rabbits, cats, dogs, and man. According to his report five rabbits, which received 0.2 gram caffein subcutaneously, eliminated in the urine from 11.1 to 21.3 per cent of the amount administered. The quantities recovered in the urine of different animals during the first twenty-four hours varied between 15 and 6.5 per cent, while during the second twenty-four hours from 1.5 to 5 per cent were found in the urine. Small amounts of caffein—1 to 4 mg—were also found in the urine at the end of seventy-two hours.

Exceptionally the elimination of caffein may continue beyond seventy-two hours. Thus an examination of the urine collected from two rabbits for five days showed the presence of larger amounts of caffein than the urine of two rabbits obtained in seventy-two hours. This, however, is not always the case, as shown by Rost in other experiments. Since the treatment of the animal may prove to be an important factor in the elimination of caffein, it may be

remarked that paraldehyde was given simultaneously when caffein was administered. Whether or not this had any effect on the elimination of caffein does not appear in the protocols to Rost's experiments, as no controls were made. The diet of these animals consisted of carrots entirely, or sometimes of carrots and bread. It would seem, therefore, that by far the greatest part was eliminated in the case of the rabbit during the first twenty-four hours; moderate amounts during the second twenty-four hours and only very small quantities during the next day.

Experiments on dogs show that these animals eliminated much smaller amounts of caffein than did rabbits. After the administration of 0.1 to 0.4 gram by mouth, 1.1 to 8.1 per cent of caffein was found in the urine of five days. Only one experiment was made by subcutaneous injection, after which 1.2 per cent of caffein was found in the urine of three days.

The amounts of caffein in the urine of cats and of man after the administration of moderately large quantities were found to be even much smaller than in that of dogs. Thus traces only were detected by Rost in the five days' urine of two cats, while 2.4 per cent of caffein was found in the case of another cat. Similar results were obtained for man. After ingesting 0.25 gram caffein, traces only were found n the urine collected in eighteen to twenty-four hours. When 0.5 gram caffein was ingested 0.45 per cent was recovered in seventeen hours' urine, and 0.6 per cent in the urine of twenty-four hours.

It appears, therefore, from the findings of Rost that the rabbit eliminated the largest amount of caffein unchanged, while in the cat and in man only minimal quantities found their way into the urine. Although the amounts of caffein reported by Rost undoubtedly represent all of the alkaloid eliminated by the kidney, as in some instances the urine of five days was examined, there is no evidence of the purity of the product obtained, as neither the melting point nor other tests, chemical or physiological, were made to identify the substance. Rost (15) assumed that the repeated extraction with alcohol, chloroform, and sodium benzoate is bound to extract caffein only.

Bongers (4) studied the elimination of caffein into the stomach; after the subcutaneous injection of 1.0 gram of caffein sodium salicylate into a dog, the examination of the contents of the stomach proved negative. When the dose was increased to 1.5 grams and administered the same way the contents of the stomach obtained half an hour later gave a distinct reaction for caffein by the method of Schwarzenbach. Alcohol, benzol, and chloroform were used for the extraction of caffein.

Later Martin Krüger (8) reported experiments on two dogs which received 50.5 grams of caffein in nearly three weeks. The urine of the

entire period, examined by an improved and more accurate method of analysis, contained 6.6 per cent of caffein, thus indicating that the dog may eliminate an appreciable quantity of caffein unchanged.

Albanese (1) examined the urine collected in thirty days from one dog which had received 42.5 grams of caffein. The alcoholic extract of the urine after evaporation was suitably treated and precipitated with phosphomolybdic acid in the presence of sulphuric acid. The precipitate was extracted with chloroform several times and crystallized. The product thus obtained, which weighed 0.5 gram, did not respond to the caffein tests. Another experiment was tried in which a dog received 3 grams of caffein in five days. Symptoms of poisoning appeared in this case. The urine was examined for caffein by Draggendorff's method, which was somewhat modified. Only traces of caffein were found in this case. Similar experiments carried out on one rabbit and on man also showed the presence in the urine of traces of a substance which was identified as caffein by mere color reactions.

According to this review of the literature, part of the caffein is eliminated unchanged in animals and in man. As the analytical methods employed, however, are far from satisfactory, the results obtained by most of the writers on this subject are not convincing. It will be noticed, for example, that no proof was brought forward of the presence of caffein other than the identification of its crystals. The melting point was not determined in any of the analyses.

The necessity of reinvestigation of the subject with special reference to the method of analysis (as well as a study of the channels of elimination of caffein) was therefore obvious. The other questions pertinent to the subject which also suggest themselves will be stated in the following chapter.

METHODS OF ANALYSIS AND PLAN OF WORK.

The method employed for the isolation of the caffein in the present research was a modification of the Draggendorff scheme, in which after clarification with lead subacetate solution the caffein was extracted with chloroform and purified by the formation of the periodid. This was decomposed with sulphurous acid and the caffein again extracted with chloroform. In this way the caffein was obtained in a state of high purity, it being possible to recover as little as 0.5 mg quantities from the fluids and tissues of the body by giving careful attention to the purity of the solvent, the proper conditions for the quantitative precipitation of the periodid, etc. The caffein thus obtained by control tests corresponded closely in melting point with pure caffein. As a further test of purity sublimation was resorted to, the beaker in which it was contained was weighed and remained clean with its original tare, so that for this

purpose the appearance of the residue to the trained eye, together with its complete sublimation, was considered sufficient for its identification and the proof of its purity. The melting point was determined in a good many cases but not in all, while sublimation was tried in every case.

The elimination of caffein was studied in rabbits, guinea pigs, in cats and dogs, the object of this investigation being the determination of the amount of caffein excreted as compared with the quantity introduced, as well as to gain some information concerning the rate of elimination, with due regard to possible factors which might influence this process. The mode of administration, the size of the dose, and diet were considered possible factors which may influence the rate of elimination. Its elimination in the urine was studied chiefly, but the contents of the various sections of the gastrointestinal canal were also examined for caffein after the drug was given.

Two, or more frequently three, animals were used for every experiment, excepting those on dogs, and the urine was combined and examined for caffein. The same procedure was employed in the case of the contents of the digestive tract or of the bile. This was done in order to make the detection of small quantities possible and to reduce individual variations by obtaining an average of several subjects in each case.

EXPERIMENTS ON RABBITS.

General Discussion.

The elimination of caffein was studied in animals on different diets, some receiving oats and some carrots, while others were given hay exclusively. The doses administered varied between 50 and 150 mg per kilo, the subcutaneous method being employed in all the experiments except one in which the drug was given by mouth.

It was found that considerable amounts of caffein were eliminated in the urine of the rabbit and that it varied appreciably, in different subjects, the difference being especially marked on oat diet. Moreover, the average amounts recovered strongly suggest that the percentage eliminated is distinctly smaller with oats than with carrots, the elimination on a diet of hay being intermediate in amount. After the subcutaneous injection of 150 mg per kilo the amounts recovered at the end of twenty-two to twenty-four hours in one series of rabbits (Series I) averaged 9.6 per cent when fed oats and 11.62 per cent when fed carrots. In another series of experiments (Series II) the results obtained were reversed, i. e., more caffein was eliminated during approximately the same time by animals that received oats than by those that were fed carrots, the percentage in the former being 12, while in the latter it was 11.23. This seemed to be exceptional,

however, as in other experiments (Series III) the total amounts of caffein recovered were 13.41 and 6.63 per cent with animals fed respectively on carrots and oats. The difference was much more striking with smaller doses (Series IV and V). After the subcutaneous administration of 50 mg per kilo from 1.72 to 5.33 per cent of caffein was obtained from the urine of rabbits fed on oats, while those fed on carrots eliminated in the urine 7.18 and 11.38 per cent.

A difference in the amounts eliminated was also observed when caffein was given by mouth. The amounts recovered from the urine were 9.5 per cent when the diet consisted of oats and 14 per cent when carrots were fed (Series VI). Diuresis suggests itself as a possible cause of the larger amounts eliminated on a carrot diet. The following experiments would seem to furnish some support for this view. In Series I rabbits fed on oats passed 250 cc of urine in three hours and eliminated 3.8 per cent of the caffein injected. Parallel experiments on animals receiving carrots showed an elimination of 4.75 per cent of the caffein administered; the amount of urine passed was 360 cc in three hours.

Two series of experiments in which hay was fed (Series VIII and IX) also indicated that diuresis favors better elimination of caffein, as the amount recovered in one series was 6 per cent and in another it was 4.8 per cent, the quantity of urine eliminated during the experimental period being 570 cc in the former and 345 cc in the latter case. Diuresis, however, is in all probability only one of the factors concerned in the elimination of caffein, as this does not always account for the differences in the amounts recovered, and it is conceivable that diet may likewise play an important part. Since the amount of caffein eliminated unchanged is an index of its decomposition in the body it follows that the greater the quantity obtained in the various channels of excretion the less the demethylation in the body. It is quite probable, therefore, that demethylation is greatest when hay is fed, less when oats, and least when carrots form the exclusive diet. In the studies on toxicity already reported (Bulletin 148) it was found, however, that the resistance to caffein was the same whether oats or carrots were fed. This may be accounted for by the fact that the toxic dose of caffein is quite large and varies a good deal for individuals of the same species, so that toxicity may be masked by these factors.

In the experiments which were made on the rate of elimination it was found that caffein disappeared from the urine within about forty-eight hours after its administration. A very small amount was found later in some experiments, but in most of them the urine obtained on the third day after the administration of caffein failed to show the presence of the alkaloid. The amounts found in the urine in the second twenty-four hours were usually small, seldom

exceeding 2 per cent, and as a rule only about 1 per cent was present, while in some cases none at all could be detected during this period, so that practically all of the caffein found in the urine is eliminated during the first twenty-four hours after its administration, the rate in all probability being greatest during the first two to three hours.

The time of the appearance of caffein in the urine has been studied in these experiments and the following data have been obtained. Its presence was detected in some animals fifteen minutes after its administration when carrots were fed. In another series of experiments with oats no caffein was found in the urine within fifteen to twenty minutes after injection. After forty minutes, however, as much as 1 per cent was recovered.

In other experiments performed in this laboratory the presence of caffein in the bile of rabbits and of other animals was detected, the bile of some animals being negative as regards caffein. It seemed desirable, therefore, to determine also whether the gastro-intestinal canal contains caffein after its subcutaneous administration. This experiment was made on two groups of rabbits, one of which was fed carrots and the other oats (Series VII). Caffein was injected subcutaneously, and the rabbits were killed 24 hours later. The amounts found in the contents of the stomach were 1.4 to 1.7 per cent of the quantity injected. In the intestinal contents the quantities varied between 1.7 for rabbits on oats and 3.56 per cent for those on carrots. The total amount found in the stomach and intestines of some rabbits was about the same as in the urine.

Series I.

In the experiments of this series 150 mg of caffein per kilo were given subcutaneously. A total of 760 mg were injected into the three rabbits which received oats. The urine secreted during the first three hours after injection contained 28.8 mg of caffein, or 3.8 per cent of the amount injected. The rabbits which were fed carrots received 830 mg of caffein. The urine of the first three hours contained 39.3 mg of caffein, or 4.75 per cent. In the next nineteen hours the amounts of caffein eliminated were somewhat more than 2 per cent in each case than in the preceding period, thus showing a much faster rate of elimination in the first period as well as a greater amount of caffein being eliminated on carrots than on oats. When the latter were fed the composite urine obtained three hours after injection was 250 cc; when carrots were fed, 360 cc. Better diuresis may explain the difference in the amount of caffein excreted.

SERIES I.—*Three rabbits (Nos. 607, 608, and 619), each receiving 150 mg of caffein per kilo subcutaneously.*

Diet and time.	Caffein recovered.		Remarks.
	Mg	Per cent.	
Oats:			
3 hours	28.8	3.80	Composite urines in three hours, 250 cc. Nos. 607 and 608 had convulsions and died during the night. No. 619, which received 240 mg caffein, was run alone for the 22-hour period. Total of 760 mg of caffein injected.
22 hours	15.0	5.80	
Total		9.60	
Carrots:			
3 hours	39.3	4.75	Composite urines in three hours, 360 cc. Total of 830 mg of caffein injected.
22 hours	57.13	6.88	
Total		11.63	

Series II.

The object of this series was to ascertain when the elimination of caffein began and the length of time during which it continued. The urines at the end of the first hour as well as at the end of 49 hours were examined for caffein. In the oat-fed rabbits 36 mg, or 4.8 per cent, were eliminated during the first hour. In the parallel experiment, in which carrots were fed, only 2 per cent of the caffein administered was found in the urine at the end of the first hour. The urine of the next 24 hours contained 7.18 and 9.23 per cent in the case of the rabbits on oats and on carrots, respectively. At the end of 49 hours the amounts of caffein recovered were 1.94 per cent in the experiment with oats and 1.29 per cent in that on carrots.

The total amounts of caffein recovered in the entire period showed that the rabbits which were fed oats eliminated about 1.5 per cent more than those fed on carrots. The loss of some of the urine in the latter case makes it probable that the difference was smaller than 1.5 per cent. The results of this series indicate, therefore, that the amounts of caffein which are eliminated unchanged may be independent of the diets employed in these experiments.

SERIES II.—*Three rabbits (No. 619 of Series I being used again); each received 150 mg per kilo subcutaneously.*

Diet and time.	Caffein recovered.		Remarks.
	Mg	Per cent.	
Oats:			
1 hour	36.00	4.87	Composite urine in 1 hour amounted to 111 cc. Total caffein injected 740 mg.
25 hours	55.18	7.18	
49 hours	14.33	1.94	
Total		13.99	
Carrots:			
1 hour	14.7	2.00	Total caffein injected, 730 mg. No. 629 found dead on second day; the 49-hour interval was continued on the other two (490 mg injected); in 25-hour interval there was loss due to overflow. Composite urines in 1 hour, 100 cc.
25 hours	67.4	9.23	
49 hours	6.3	1.29	
Total		12.52	

Series III.

In these experiments the urine was obtained 15 to 20 minutes after the injection of caffein. By reference to the table it will be noticed that none was found at this time in the urine of the rabbits which were fed oats, while only a little more than a trace was detected in the urine of the rabbits fed on carrots. The elimination of caffein does not begin, therefore, so soon as this after its subcutaneous injection as in the case of other alkaloids, like strychnin. The examination of the urine of rabbit No. 651 shows, however, that 1.04 per cent of caffein was recovered from the urine passed 40 minutes after injection. During the next 40 minutes this rabbit eliminated 1.25 per cent more caffein, making a total of 2.25 per cent in 80 minutes, while rabbit No. 652 of the same group eliminated slightly more than 2 per cent in 95 minutes and rabbit No. 653 eliminated 1.86 per cent in 75 minutes.

The rate of elimination of caffein also differed but little during the next twenty-six hours in these cases, being 5.11 per cent for No. 651, 5.33 per cent for No. 652, and 4 per cent in No. 653. Analysis of the urine of the following twenty-four hours shows that elimination of caffein has been completed at this time, since no caffein was found. The total amounts of caffein eliminated in the case of the rabbits which were fed carrots was 11.63 per cent at the end of forty-two hours, while in the urine of the next twenty-four hours 1.65 per cent of caffein was found.

SERIES III.—*Experiment A.—Oats diet, 150 mg of caffein per kilo injected.*

Rabbit No. 651. Weight, 1,635 grams. Received 12 cc of 2 per cent caffein subcutaneously on June 8, 10.35 a. m.

Caffein recovered.	Date and time.	Remarks.
Per cent.	June 8.	
None.	10.50 a. m.	Bladder squeezed and urine of the 3 rabbits composited (2 cc).
1.04	11.15 a. m.	Urinated 20 cc lightly colored urine.
1.25	11.55 a. m.	Urinated 25 cc slightly colored urine.
	June 9.	
5.11	2.00 p. m.	Bladder squeezed and cage washed.
Trace.	10.00 p. m.	Bladder squeezed. Composited.
	June 10.	
Trace.	10.00 a. m.	Do.
Total 7.40	47.5 hours.	

SERIES III.—*Experiment A.—Oats diet, 150 mg of caffein per kilo injected*—Continued.

Rabbit No. 652. Weight, 1,955 grams. Received 15 cc of 2 per cent caffein subcutaneously on June 8, 10.50 a. m.

None.	June 8. 11.05 a. m.	Bladder squeezed and urine of the three rabbits composited (2 cc).
2.08	12.25 p. m.	Urinated 40 cc of light colored urine.
5.33	June 9. 2.00 p. m.	Bladder squeezed and cage washed.
	10.00 p. m.	Found dead. Autopsy: lungs and liver congested and the blood vessels of the large intestine injected.
Total 7.41	27 hours.	

Rabbit No. 653. Weight, 1,615 grams. Received 2 per cent caffein subcutaneously on June 8, 11.05 a. m.

None.	June 8. 11.25 a. m.	Bladder squeezed and urine composited (3 cc).
1.86	12.20 p. m.	Urinated 25 cc.
4.00	June 9. 2.00 p. m.	Bladder squeezed and cage washed.
5.86	.....	
Trace.	10.00 p. m.	Bladder squeezed.
Trace.	June 10. 10.00 p. m.	Bladder squeezed and cage washed.
Total 5.86	47 hours.	

Experiment B.—Carrot diet, 150 mg of caffein per kilo injected.

White rabbit No. 621, weight 1,685 grams, had been used for two similar experiments; white rabbit No. 610, weight 1,485 grams, had been used for two similar experiments; white rabbit No. 654, weight 2,400 grams, new subject, pregnant.

No. 621 urinated 15 minutes after injection and 0.125 per cent of the caffein administered was recovered. In the composite urines of the three rabbits, collected forty-two hours after injection, 11.63 per cent of the caffein injected was recovered, while in the urine collected at the end of sixty-six hours 1.65 per cent was recovered, making a total of 13.28 per cent eliminated in sixty-six hours.

Series IV.

The elimination of caffein when small doses are given were studied in these experiments. Fifty milligrams per kilo were injected into each of the rabbits of this series. The difference in the amounts recovered in the two groups of rabbits was very striking. Elimination was complete in the rabbits fed on oats, as well as those on a carrot diet, at the end of twenty-four hours. The larger amounts of caffein recovered in the latter case may be due to the greater quantity of urine passed or there may be a compensatory factor in the case of the rabbits fed on oats, retarded elimination in the urine being caused by the increased excretion into the gastrointestinal canal. As will be seen later, the excretion of caffein into the stomach and intestines is greater on an oat diet than on a diet of carrots, but as it is reabsorbed into circulation it ought to be found in the urine ultimately. The elimination of larger amounts of caffein into the gastrointestinal canal is not the cause therefore of the small amounts of it found in the urine when oats were fed. Demethylation is probably increased when smaller doses are administered.

SERIES IV.—*Rabbits to which 50 mg caffein per kilo were administered subcutaneously.*

Rabbit No. and diet.	Weight.	Urine data for—					
		3 hours.		24 hours.		72 hours.	
		Volume.	Caffein recovered.	Volume.	Caffein recovered.	Volume.	Caffein recovered.[1]
Oats:	*Grams.*	*cc*	*Per cent.*	*cc*	*Per cent.*	*cc*	*Per cent.*
658	1,650	20	1.20	75	0.52	95	Trace.
657	1,445	30		80		95	
651	1,650	60		55		95	
Carrots:							
621	1,655	[2] 75	2.70	410	4.48	320	None.
638	1,745	[2] 68		260		400	
639	1,625	[2] 60		240		350	

[1] Total recovery for oat fed rabbits 1.72 per cent, for carrot fed 7.18 per cent.
[2] In 2 hours.

Series V.

SERIES V.—*Rabbits receiving 50 mg per kilo of caffein.*

Rabbit No. and diet.	Weight.	Time.	Food consumed.	Water.	Volume of urine.	Caffein recovered from composite sample.	
						Time.	Amount.
Oats:	*Grams.*	*Hrs.*	*Grams.*	*cc*	*cc*	*Hrs.*	*Per cent.*
724	2,265	3.5	.....	.....	40		
		24.0	100	140	45		
		48.0	125	100	35		
		72.0	45	None.	45		
725	2,480	3.5	.....	.....	100	3.5	3.00
		24.0	95	200	95	24.0	2.33
		48.0	80	100	80	48.0	Trace.
		72.0	75	100	80	72.0	None.
726	1,620	3.5	.....	.....	20		
		24.0	65	135	Lost.		
		48.0	75	100	30		
		72.0	50	100	15		
Total	.....	.....	.....	.....	.....	.....	5.33
Carrots:							
727[1]	1,900	3.5	.....	.....	55		
		24.0	500	None.	275		
		48.0	500	30	220		
		72.0	400	None.	165		
728[1]	1,740	3.5	.....	.....	75	3.5	3.55
		24.0	500	None.	240	24.0	7.83
		48.0	500	None.	270	48.0	Trace.
		72.0	500	None.	265	72.0	None.
729	2,030	3.5	.....	.....	85		
		24.0	500	None.	230		
		48.0	500	None.	250		
		72.0	500	None.	250		
Total	.....	.....	.....	.....	.....	.....	11.38

[1] Small amount of feces excreted by these rabbits, probably not over 50 grams during the entire experiment; there was no diarrhea whatever.

The enormous difference in the amounts of caffein eliminated on the diets employed in the last series made the repetition of these experiments necessary. Although considerably more caffein was excreted on an oat diet than in Series IV much less was recovered than in the parallel experiments when feeding carrots. It will be noticed also that the appetite was not impaired and that much larger amounts of urine were passed when carrots were eaten.

Series VI.

The rabbits in these experiments were fed caffein by mouth. The feces as well as the urine were examined for caffein. It will be noticed that the weights of the rabbits employed in these experiments differed but little, all of them being of medium size and of the same age. The amounts of caffein eliminated by the two groups of rabbits differed considerably, as at the end of twenty-four hours a little over 13 per cent of caffein was found in the urine of the rabbits fed on carrots, while those on oats eliminated in the urine during the same time only 8.02 per cent. The amounts of caffein recovered from the urine during the next twenty-four hours were 1 per cent for those fed on carrots and 0.9 per cent for those which were given oats. Although the latter continued to eliminate caffein in the urine during the following 24-hour period the amount was rather small. None was found in the urine of the other rabbit during this period. It may be concluded, therefore, that the elimination of caffein is practically completed within forty-eight hours and does not continue beyond this time. Examination of the feces indicates the presence of appreciable amounts of caffein, which is probably due to excretion into the gastrointestinal canal as well as to lack of absorption. It will be noticed that the rabbits which received oats eliminated apparently more caffein in the feces than those whose diet consisted of carrots, which is in all probability due to the difference in the elimination of caffein by the gastric and intestinal epithelium on an oat than on a carrot diet.

SERIES VI.—*Rabbits to which 150 mg of caffein per kilo were administered by mouth.*

Rabbit No. and diet.	Weight.	Time.	Amount eaten.	Urine.	Feces.	Caffein recovered in composite sample.	
						In urine.	In feces.
Carrots:	*Grams.*	*Hrs.*	*Grams.*	*cc*	*Grams.*		
710	1,485	1	.....	40	.....		
	1,385	24	130	180	.....		
	1,440	48	300	150	10		
	1,520	72	450	300	.....		
711	1,660	1	.....	40	.....	1.025	.....
	1,580	24	200	210	.....	12.000	0.60
	1,640	48	300	175	10	1.000	.20
	1,700	72	400	225	.....	0.000	.00
712	1,400	1	.....	40	.....		
	1,390	24	250	240	10		
	1,405	48	300	200	10		
	1,465	72	350	300	.....		
Total	.....	.....	.....	2,100	40	14.025	.80
						14.825	
Oats:							
713	1,265	24	Fair.	(?)175	20		
	1,240	48	Fair.	75	25		
	1,225	72	Good.	75	25		
714	1,285	24	None.	90	50	8.02	.934
	1,240	48	Some.	50	.....	.90	.300
	1,370	72	Some.	Lost.	10	.56	.200
715	1,370	24	None.	115	10		
	1,205	48	None.	25	.....		
	1,150	72	None.	25	.....		
Total	.....	.....	.....	630	140	9.48	1.434
						10.914	

Series VII.

The object of these experiments was the study of the elimination of caffein by the kidney and into the gastrointestinal canal. The contents of the stomach and intestines were carefully removed and separately examined for caffein, while the feces passed after the injection of caffein until the death of the animals were collected and the amount of caffein determined. Only small quantities—1.2 to 2 mg—were found. The intestinal contents, however, contained appreciable quantities of caffein. The rabbits on the oat diet eliminated 19.3 mg, or 3.56 per cent, while for those on a diet of carrots only 1.7 per cent of the caffein ingested was recovered, little difference being observed in the percentage, as well as the absolute amounts of caffein recovered from the gastric contents of both groups of rabbits. Two groups of rabbits were employed, three being fed on oats and three on carrots. Twenty-four hours after having received 150 mg of caffein per kilo subcutaneously the animals were killed and the urine, feces, intestinal contents, and stomach contents were examined separately, with the following results:

SERIES VII.—*Post-mortem examination of rabbits 24 hours after injecting 150 mg of caffein per kilo.*

Rabbit No. and diet.	Weight.	Volume of urine.	Food consumed.	Water.
Carrots:	*Grams.*	*cc*	*Grams.*	*cc*
704	1,730	155	None.	25
705	1,425	130	None.	25
706	1,665	195	80	50
Oats:				
703	1,620	95	Some.	25
707	1,295	130	None.	50
708	1,445	75	None.	None.

CAFFEIN RECOVERED.

Data.	Rabbits on carrot diet—720 mg caffein injected.		Rabbits on oat diet—660 mg caffein injected.	
	mg	*Per cent.*	*mg*	*Per cent.*
In urine	54	7.5	31.3	4.75
Intestinal contents	12	1.7	19.3	3.56
Stomach contents	10	1.4	11.2	1.70
Feces	2	0.3	1.2	0.20
Total	78	10.9	63.0	10.21

Series VIII and IX.

These rabbits were fed hay exclusively for several days before and after the administration of caffein. In both experiments nearly the entire amount of caffein eliminated was found in the urine of the first twenty-four hours. The diuretic effect of caffein was marked in all of the rabbits except in No. 723 of Series IX, in which the amount of

urine passed was the same during the twenty-four hours after the administration of caffein as in the following period. It is noteworthy that in this series the percentage amount of caffein eliminated was less than in Series VIII. The differences in the amount of urine passed may account, therefore, for the relatively smaller quantity of caffein eliminated in Series IX.

SERIES VIII and IX.—*Duplicate experiments on two groups of five rabbits on a hay diet, given 100 mg caffein per kilo (Nov. 25–30).*

No. and series.	Time.	Weight.	Diet.		Volume of urine.	Caffein eliminated in composite samples.
			Water.	Hay.		
Series VIII:	*Hours.*	*Grams.*	*cc*	*Grams.*	*cc*	*Per cent.*
718	24	2,040	300	125	215	
	48	.....	200	125	100	
	72	.....	200	125	100	
719	24	1,685	300	125	185	5.96
	48	.....	175	125	120	.10
	72	.....	200	50	90	None.
720	24	1,565	300	125	170	
	48	.....	200	125	95	
	72	.....	200	125	110	
Total	.....	.....	.....	.....	.....	6.06
Series IX:						
722	24	1,840	300	125	255	
	48	.....	200	125	135	4.76
	72	.....	200	50	130	.10
723	24	1,900	300	125	90	None.
	48	.....	125	125	105	
	72	.....	200	100	65	
Total	.....	.....	.....	.....	.....	4.86

EXPERIMENTS ON GUINEA PIGS (SERIES X AND XI).

The elimination of caffein in the case of the guinea pig was studied in four series of experiments, in two of which the animals were fed oats while the other two received carrots. The amounts of caffein eliminated varied considerably, 8.43 and 6.36 per cent caffein being found in the first twenty-four hours' urine of guinea pigs fed on carrots, while those on oats eliminated 4.84 and 5 per cent. In series X the enormous difference in the amount of urine passed by the carrot and oat-fed rabbits and the absence of increased diuresis is of interest in this connection. In the second twenty-four hours much smaller quantities of caffein were found; as shown in the table for series XI, the guinea pigs eating carrots eliminated 1.56 per cent; those on a diet of oats eliminated only 0.55 per cent. There was considerable elimination into the gastrointestinal tract during the first twenty-four hours, almost twice as much on the oats diet as on carrots. This is probably due to better diuresis when carrots were eaten. Examination of the gastrointestinal contents and feces at the end of forty-eight hours showed the presence of small quantities of caffein—only 0.5 per cent of the amount injected being recovered in each of the two experi-

ments. This disappearance of caffein from the stomach and intestines may be due to bacterial decomposition or to reabsorption. But since caffein is known to resist putrefactive changes, absorption from the gastrointestinal tract and decomposition in the tissues must be assumed, which may account for the smaller percentage amounts of caffein found at the end of forty-eight hours.

SERIES X.—*Guinea pigs, 100 mg per kilo of caffein injected, 24-hour period.*

No. and diet.	Weight.		Food consumed.	Water.	Excreta.		Caffein recovered from composite samples.	
	Initial.	Final.			Urine.	Feces.	Urine.	Gastrointestinal tract.
Carrots:	*Grams.*	*Grams.*	*Grams.*	*cc*	*cc*	*Grams.*	*Per cent.*	*Per cent.*
150	620	560	None.	None.	45	None.	.....	.....
151	560	525	50	40	60	None.	8.43	2.65
152	555	535	40	20	60	None.	.....	.....
Total	.....	.....	90	60	165	.....	11.08	
Oats:								
153	375	355	10	10	15	10	.....	.....
154	430	405	10	10	10	10	4.84	5.0
155	365	345	None.	None.	10	10	.....	.....
Total	.....	.....	20	20	35	30	9.84	

SERIES XI.—*Guinea pigs, 100 mg per kilo of caffein injected, 48-hour period.*

No. and diet.	Time.	Weight.	Food consumed.	Water.	Excreta.		Caffein recovered from composite samples.	
					Urine.	Feces.	Urine.	Gastrointestinal tract.
Carrots:	*Hours.*	*Grams.*	*Grams.*	*cc*	*cc*	*Grams.*	*Per cent.*	*Per cent.*
162	Initial.	430	.....	.....	.....	.....	.....	.....
	24	385	.....	.....	20	.....	.....	.....
	48	365	.....	.....	10	.....	.....	.....
157	Initial.	440	.....	.....	.....	.....	6.36	.....
	24	415	40	.....	20	.....	1.56	0.5
	48	415	75	.....	50	5	.....	.....
158	Initial.	425	.....	.....	.....	.....	.....	.....
	24	395	50	10	40	10	.....	.....
	48	400	100	.....	65	10	.....	.....
Total	.....	.....	265	10	205	25	8.42	
Oats:								
159	Initial.	625	.....	.....	.....	.....	.....	.....
	24	600	15	10	15	15	.....	.....
	48	590	5	15	20	10	.....	.....
160	Initial.	720	.....	.....	.....	.....	5.00	.....
	24	670	.....	.....	20	10	.55	0.50
	48	650	.....	.....	20	.....	.....	.....
161	Initial.	840	.....	.....	.....	.....	.....	.....
	24	775	.....	20	55	10	.....	.....
	48	755	20	15	25	.....	.....	.....
Total	.....	.....	40	60	155	45	6.05	

EXPERIMENTS ON CATS AND DOGS.

Experiments on the elimination of caffein were also carried out on cats and dogs. The results show the interesting fact that the elimination in these animals is much less than in rabbits or in guinea pigs. Only 1.3 per cent was recovered in the urine of a dog during twenty-four hours, and the same amount was recovered in the urine and the gastrointestinal canal of cats. It might also be added that small quantities of caffein were found in the urine and bile of dogs two or three hours after its intravenous injection. Moreover, the amounts found were the same in the bile as in the urine, thus indicating that soon after its introduction into the blood stream the liver is as efficient an organ for the elimination of caffein as are the kidneys.

Caffein Elimination in Dogs.

Dog No. 83.—Weight, 5.6 kilos; had been given 1.6 grams caffein intravenously in the course of a blood-pressure experiment; gall bladder contents, 15 cc, 0.9 mg of caffein recovered; bladder contents, 15 cc, 1.0 mg of caffein recovered.

Dog No. 84.—Female fox terrier; weight, 5.250 kilos.

November 22, 2.15 p. m., received 26 cc of 2 per cent caffein subcutaneously or 100 mg per kilo; 4.15 p. m., catheterized, obtained 15 cc urine; total urine, 215 cc, in which 0.65 per cent of the caffein was recovered; had vomited and defecated during this interval.

November 23, 2.15 p. m., catheterized, urine 20 cc; total urine 95 cc, in which 0.65 per cent of the caffein was recovered.

November 24, 2.15 p. m., urine collected, amount 300 cc, showing a trace only of caffein; total caffein recovered was 1.30 per cent.

Dog No. 85.—Weight, 21.5 kilos; had been given 1.5 grams caffein intravenously in the course of a blood pressure experiment; gall bladder contents, 25 cc, contained 2 mg of caffein; bladder contents, 75 cc, contained 1 mg of caffein.

Caffein Elimination in Cats when 100 mg were Injected Subcutaneously (Nov. 12, 1911).

No. 87.—Weight, 2,645 grams; meat eaten, 100 grams; water, 15 cc; urine, 45 cc.
No. 98.—Weight, 2.855 grams; meat eaten, none; water, none; urine, 15 cc.
No. 99.—Weight, 2,920 grams; meat, 50 grams; water, 15 cc; urine, 50 cc.

Caffein recovered twenty-four hours after injection, from composite sample.

	Per cent.
Urine (7.5 mg)	0.888
Feces (2.1 mg)	.247
Stomach (1.2 mg)	.140
Intestines (1.2 mg)	.140
Bile	.000
Total	1.415

The gall bladders of the three animals gave 25 cc of bile; the stomach of cat No. 87 was full; the stomachs of the other cats were empty.

SUMMARY AND CONCLUSIONS.

Caffein administered subcutaneously, by mouth, or intravenously is eliminated in part unchanged, in the urine, into the gastrointestinal canal, and into the bile. The amounts recovered in the urine of rabbits varied from 1.72 to 14.02 per cent of the quantity introduced into the body. In most cases it was approximately 6 to 10 per cent. More caffein was eliminated on a diet of carrots than on oats or hay, which is probably due in part to the increased diuresis when carrots are eaten. The elimination of caffein in the guinea pig was found to be between 6 and 11 per cent. As in the rabbit, more caffein was found in the urine of carrot-fed subjects as compared with the oat fed. The elimination into the gastrointestinal canal was found to be marked in the guinea pigs as well as in rabbits. In both cases more caffein was found when oats were fed than when a diet of carrots was given. The presence of very small quantities of caffein in the gastrointestinal contents of animals at the end of forty-eight hours points to its reabsorption into the circulation, since destruction of caffein is highly improbable on account of its resistance to bacterial action.

Cats and dogs eliminated very small quantities, slightly over 1 per cent of the amount ingested. The elimination of caffein begins soon after its introduction into the circulation. It was found in the urine from fifteen to forty minutes after its subcutaneous injection and in some cases continued to be present for forty-eight hours. The greater part, however, is eliminated during the first twenty-four hours, only small quantities being found in the urine later.

The data herein presented lead to the conclusion that in the carnivora larger amounts of caffein are demethylated than in the herbivora, and that the resistance to caffein is inversely as demethylation, since it has been shown that caffein is much more toxic for carnivora than for herbivora. The mechanism of demethylation is in all probability utilized in the body as a means of defense against the deleterious action of caffein, being more active in organisms for which the drug is more toxic.

Summary of data on elimination of caffein.

RABBITS.

Series.	Mg caffein per kilo.	Caffein eliminated. First period.	Second period.	Third period.	Total.	Remarks.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	
1 Oats	150	*3 hrs.* 3.8	*22 hrs.* 5.8	.....	9.6	250 cc urine in 3 hours.
1 Carrots	150	4.75	6.88	.....	11.63	360 cc urine in 3 hours.
2 Oats	150	*1 hr.* 4.87	*25 hrs.* 7.18	*49 hrs.* 1.94	13.99	111 cc urine in 1 hour.
2 Carrots	150	2	9.23	1.29	12.52	100 cc urine in 1 hour.
3 651, oats	150	*40 min.* 1.04	*80 min.* 1.25	*27½ hrs.* 5.11	7.4	
3 652, oats	150	*95 min.* 2.08	*27 hrs.* 5.33	.....	7.38	
3 653, oats	150	*75 min.* 1.86	*27 hrs.* 4	*47 hrs.* None.	5.86	
3 Carrots	150	*15 min.* 0.125	*42 hrs.* 11.63	*66 hrs.* 1.65	13.28	
4 Oats	50	*3 hrs.* 1.2	*24 hrs.* 0.52	.....	1.72	320 cc urine in 24 hours.
4 Carrots	50	*2 hrs.* 2.7	*24 hrs.* 4.48	.....	7.18	1,113 cc urine in 24 hours.
5 Oats	50	*3½ hrs.* 3	*24 hrs.* 2.33	*48 hrs.* Trace.	5.33	300 cc urine in 24 hours. (Some lost, see protocol.)
5 Carrots	50	*3½ hrs.* 3.55	*24 hrs.* 7.83	*48 hrs.* Trace.	7.83	960 cc urine in 24 hours.
6 Oats	150	*24 hrs.* 8	*48 hrs.* 0.9	*72 hrs.* 0.56	9.48	Urine, 380 cc in 24 hours.
		0.934	.3	.2	1.434	Feces.
6 Carrots	[1] 150	*1 hr.* 1.025	*24 hrs.* 12	*48 hrs.* 1	14.025	Urine, 750 cc in 25 hours.
			0.6	0.2	.8	Feces.
7 Oats	150	*24 hrs.* 4.75	.....	.....	10.21	300 cc urine.
		1.7	.....	.....		Stomach.
		3.56	.....	.....		Intestine.
		.2	.....	.....		Feces.
7 Carrots	150	7.5	.....	.....	10.90	480 cc urine.
		1.4	.....	.....		Stomach.
		1.7	.....	.....		Intestine.
		.3	.....	.....		Feces.
8 Hay	100	*24 hrs.* 5.96	*48 hrs.* 0.1	*72 hrs.* None.	6.06	Average urine, 390 cc per rabbit.
9 Hay	100	*24 hrs.* 4.76	*48 hrs.* 0.1	*72 hrs.* None.	4.86	Average urine, 395 cc per rabbit.

GUINEA PIGS.

10 Oats	100	*24 hrs.* 4.84	.....	.....	9.84	35 cc urine, gastrointestinal canal.
		5	.....	.....		
10 Carrots	100	8.43	.....	.....	11.08	165 cc urine, gastrointestinal canal.
		2.65	.....	.....		
11 Oats	100	*24 hrs.* 5	*48 hrs.* 0.55	.....	6.05	90 cc urine in 24 hours, gastrointestinal canal.
		.....	.5	.....		
11 Carrots	100	6.36	1.56	.....	8.42	80 cc urine in 24 hours, gastrointestinal canal.
		.....	.5	.....		

[1] 72-hour period run, no caffein recovered in urine or feces.

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